Recognition of a new species of *Pomaderris* (Rhamnaceae) in eastern Victoria based on morphological and chemical data

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Abstract

Pomaderris prunifolia A.Cunn. ex Fenzl is a variable and widespread species in south eastern Australia. Within the current circumscription of this species is a variant recognised by its distinctive leaf morphology. This study has determined that this variant should be recognized as a separate species, based on both leaf morphology and phenolics. The study of morphology employed both conventional hand measured methods and landmark shape analysis. The new species, *Pomaderris briagolensis* Messina is described and illustrated, and its conservation status discussed.

Keywords: taxonomy, *Pomaderris*, Rhamnaceae, morphometrics, Victoria.

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Introduction

The genus *Pomaderris* (Rhamnaceae) is centered in Australia, where, at present, 68 species are recognised, five of which are also found in New Zealand. A further three species are endemic to New Zealand (Moore 1986; Walsh 1999, 2008, in ed.). This genus is considered an old group in the Australian flora, distributed across south-western, southern and eastern Australia, and is most diverse in eastern Australia (Ladiges *et al.* 2005). Much of the taxonomy of this genus has been clarified recently, with numerous new species described from eastern Australia (Walsh 1988, 1989, 1990a, 1990b, 1992, 2008; Ross, 1990; Walsh & Coates 1997; Millott & McDougall 2005; Kellermann *et al.* 2006). Many of these studies have identified rare, and/or narrowly endemic species, particularly in south-eastern New South Wales and eastern Victoria.

Pomaderris prunifolia A.Cunn. ex Fenzl is distributed through eastern Australia, mainly east of the Dividing Range, from Stanthorpe in south-eastern Queensland, south to the Grampians in western Victoria. There are currently two recognised varieties of *P. prunifolia*; var. *prunifolia* in Australia and var. *edgerlyi* (Hook.f.) L.B. Moore, a prostrate shrub endemic to New Zealand (Allan 1961). The distinctive habit of var. *edgerlyi*, and its wide geographic separation from the typical variety suggests an elevation in taxonomic rank is warranted, but it was not included in the present study.

This study investigates a population currently referred to as *Pomaderris* sp. aff. *prunifolia* (Briagolong) (Walsh & Stajsic 2007), and mentioned in a note under *P. prunifolia* var. *prunifolia* in Walsh (1999). It shares with *P. prunifolia* generally similar indumentum and floral characters but is reported to differ from it, particularly in leaf shape, size, margin features and texture. At present this variant is only known to occur in a single catchment area near Briagolong in eastern Victoria. At one location, both 'typical' *P. prunifolia* and the Briagolong variant

occur together with no intermediates having been observed. This study aims to investigate whether the variant warrants formal taxonomic recognition, and if so, at what level it is most appropriately recognised. For simplicity, it is referred to hereafter in this paper as the 'Briagolong variant'.

In establishing whether the Briagolong variant diverges significantly from specimens referred to the widespread, variable *P. prunifolia*, several techniques were used. These included a range of morphological analysis techniques, and chemical (flavonoid) analysis of the leaves.

Over the past two decades, several methods for describing shapes mathematically utilising advances in computer technology have been developed as an alternative to using traditional morphological methods, such as linear measures of length and width. These computer-based methods appear to have great potential for analysis of leaf shape in aiding species delimitation, especially where leaf shape is an apparently strong character, such as in some species of *Pomaderris* (e.g. Walsh 1999). These methods are capable of rapidly describing complex shapes in great detail, allowing characters that are usually difficult to measure by hand to be easily included in analysis (West & Noble 1984; Ferson *et al.* 1985). Along with a traditional morphometric, multi-character analysis of herbarium specimens, computer visualisation of leaf shapes using both a traditional linear analysis, and a landmark analysis technique developed by Rohlf (2003) were employed to detect any distinct entities within *P. prunifolia*. As well as corroborating the morphometric analysis, the shape analyses were used to test the assertion that as landmark-type techniques include more complex measures in analysis they will produce more accurate results as shown by Kincaid and Schneider (1982), West and Noble (1984) and Ferson *et al.* (1985).

Landmark analysis is similar to traditional analysis, as linear measurements are made from designated points. However, in this method, multiple measures are used simultaneously, rather than one at a time (McLellan & Endler 1998). This method also differs from traditional analysis as the relative positioning of landmarks is measured rather than the simple distance between landmarks (Jensen 1990). Landmarks are based on biologically homologous points on leaves. This allows biological relationships to be established relatively easily from data (Hammer 2002; Jensen *et al.* 2002). Landmark analysis can be correlated to original measurements, allowing highly localised changes in shape to be detected. Consequently, differences in certain structures are detected, rather than broad differences in overall shape (Rohlf & Marcus 1993).

Methods

Herbarium specimens, along with freshly collected material were used in this study. Morphology was analysed by two methods; firstly by traditional manually measured analysis of overall morphology based on herbarium material across the range of the species, and also by computer-based morphometric analysis of leaves, using freshly collected material. An analysis of leaf flavonoids was also performed using freshly collected material. Seedlings were grown in uniform glasshouse conditions at the La Trobe University, Bundoora, Victoria. Field observations and herbarium database records were used for additional information. Collection sites were derived from herbarium specimens and further field searches.

Collections

For landmark and flavonoid analysis populations of *P. prunifolia* were sampled throughout Victoria across a wide range of habitat types in an attempt to cover the range of variation in this species (Appendix 1). Victoria contains populations in most broad habitat types in which *P. prunifolia* occurs, from coastal areas through to sub-alpine areas and from dry to mesic forest types. The Briagolong variant was collected from five sites along Freestone Creek, north-west of Briagolong township. These represent the only known localities of the entity. Voucher specimens of collections were lodged at the La Trobe University herbarium (LTB).

Morphology

Hand measured data

Quantitative and qualitative measurements were manually scored using herbarium material; these measurements included length and width measurements of organs as well as derived ratios, number of veins and flowers, and ordered qualitative measures including shape and texture measures (Table 1).

Table 1. List of manually scored morphological characters.

Character type	Character description
Qualitative	leaf shape (SHAPE): ovate (0), elliptic (1), obovate (2)
	leaf margin (MARG): toothed (0), sinuate (1), entire(2)
	leaf apex (APEX): acute (0), obtuse (1), emarginated (2)
	adaxial leaf hair distribution (HAIRD): dense cover (0), low-moderate cover (1), absent (2)
	abaxial leaf hair type (HAIRB): stellate (0), stellate and simple on veins (1), stellate and simple all over (2)
Quantitative	leaf length (LLEN): mm
	leaf width (LWID): mm
	number of veins (VEINS): numeric
	stipule length (STIPL): mm
Derived ratios	leaf width/leaf length (LWTOL)
	number of veins/leaf length (VETOL)

The five largest leaves of each specimen were chosen for analysis, ensuring as much as possible that only mature leaves were selected. The mean of these five leaves was used for numerical analysis. The mean of two inflorescences per specimen was used to calculate floral characters, as most specimens contained fewer than five inflorescences. All specimens held by the National Herbarium of Victoria (MEL) and subsequent fresh collections were analysed. A list of these specimens is available from the authors.

Computer-based analysis

From each field collection, the ten best (most intact) leaves were removed from the point of attachment of the petiole and stem of five branches per population. Leaves were kept flat between sheets of cardboard for 24 hours before taking a black and white photocopy of the adaxial surface of each set of fifty leaves. This photocopy was then scanned into a computer using a CanoScan N670U scanner at 400dpi resolution. Adobe Photoshop v.7.0 was used to manipulate the images; making outlines clear by adjusting the image threshold, and arranging them vertically.

Traditional analysis was performed using silhouettes obtained using Photoshop. These were printed and captured by the program MorphoSys v. 1.26 (Meacham & Duncan 1991) using a Mintron CCD video camera. On each image six points were marked; 1 — petiole tip, 2 and 6 — petiole attachment to lamina surface, 3 and 5 — widest point, and 4 — the leaf tip. These points were marked to allow leaf area (AREA), perimeter (PERIM), lamina length (LLEN), lamina width (LWID), distance to widest point (DWID), petiole length (PETI), leaf length to width ratio (LL:W), distance to widest point to length ratio (LD:L), and degree of leaf curvature (CURV) to be measured. MorphoSys records leaf outlines as a series of chain codes. These were transformed into XY coordinates for use later. A program was written (T. Whiffin unpubl.) to perform this transformation into XY coordinates, with landmarks two and six being the start and end point of the outline (removing the petiole).

Landmark configurations recorded in MorphoSys were also used in landmark analysis by tpsSuper (Rohlf 2003). The tpsSuper program superimposes leaf images on each other, so before analysis leaf images were manipulated so that they were lying flat on the X axis. A further program was written (T. Whiffin unpbl.) to align the leaf apex (landmark 3) on the X axis, putting the leaf base (the point between landmarks 1 and 5) on the other end of the X axis. Landmark 1 was not included in this analysis as the procedure analyses lamina shape. Once images were aligned on the X axis, tpsSuper was used to obtain a landmark consensus configuration for each individual. Text files produced for plant configurations were grouped together into populations and saved as .tps files. These were re-entered into tpsSuper giving a consensus shape analysis for each population.

Flavonoid analysis

Flavonoid analysis was performed using leaves of five individuals per population. Depending on leaf size,

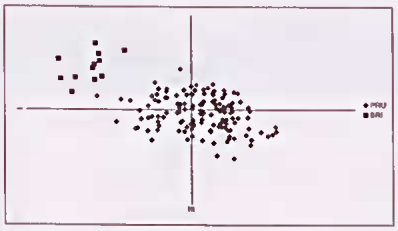

Figure 1: Ordination of hand measured morphological data (GM PCA). Stress: 0.109. Axis I and III shown.

between ten and twenty leaves were collected from each of the five individuals and air-dried in paper bags. Leaf material used in the morphological and chemical analysis was taken from the same plants. Only fresh material was used in this study, as herbarium material does not always give satisfactory results. Fungal action and oxidization during drying stages can change some of the chemical constituents found in old leaves (Markham 1982). Flavonoid extraction and separation techniques followed the procedures of Mabry *et al.* (1970) and Markham (1982). Leaf extracts were run in two dimensions on Whatman 3MM (46 X 57 cm) chromatography paper, firstly using t-butanol:acetic acid:water 3:1:1 (TBA) and secondly using 15% aqueous acetic acid (HOAc).

Once dried, phenolic compounds were viewed using long-wavelength ultraviolet light. Ammonia vapor was used to further enhance spot differentiation. Relevant spots were marked and given a number based on their relative position and colour, as seen on composite sheets. Each population was then scored for presence or absence of each constituent. No attempt was made to chemically characterise any compound, or to determine quantitative amounts of compounds.

Numerical analysis of morphology and phenolics

Multivariate analysis was performed to analyse data using PATN (Belbin 1987). Cluster and ordination analysis were performed on range-standardised data. The distance matrix was calculated using the Gower Metric (GM). This was input into cluster analysis using unweighted pair-group method using arithmetic averages (UPGMA) to produce dendrograms. Ordinations were performed using principal coordinates analysis (PCA).

Results and Discussion

Morphology

Hand measured data

Manually measured morphological characters are able to separate the Briagolong variant from typical *P. prunifolia* (Fig. 1). There is one individual of *P. prunifolia* that approaches the cluster of the Briagolong variant specimens. This specimen represented the highest recorded altitudinal occurrence of the species, (near Mt Arbuckle at approximately 1300m), and it could be anticipated that it possessed atypical characteristics. It is a small leaved specimen, presumably a reflection of the exposed nature of the site. In other respects (e.g. leaf shape, upper surface texture) it did not visually match specimens of the Briagolong variant. This analysis only included vegetative characters as initial assessment of the characters showed insufficient variation within the dataset to separate the two entities. This is not an uncommon situation in closely related species of *Pomaderris* (e.g. Walsh 1999).

Leaf dimension and shape data

Using MorphoSys to generate linear measures of leaf shape found three main clusters, again showing populations of the two entities to be separate. However this analysis split the typical form of *P. prunifolia* into two groups, with one group clustering more closely with the Briagolong variant (Fig. 2). Here the problems with using size as a surrogate of shape can be seen as *P. prunifolia* cannot be separated from the Briagolong variant based on linear measures alone. Furthermore, this result may not be biologically meaningful, as leaf size is generally not thought to be independent of environment (Kincaid & Schneider 1982; Dickinson *et al.* 1987; Rohlf & Bookstein 1987).

Landmark analysis

Pomaderris prunifolia and the Briagolong variant are shown to be very distinct groups by landmark TPS analysis (Fig. 3). Multivariate analysis shows all populations of the Briagolong variant to be very similar to each other, and different from *P. prunifolia*. This supports the contention that the Briagolong variant deserves taxonomic recognition based on leaf shape, as there is non-overlapping variation between it and *P. prunifolia*.

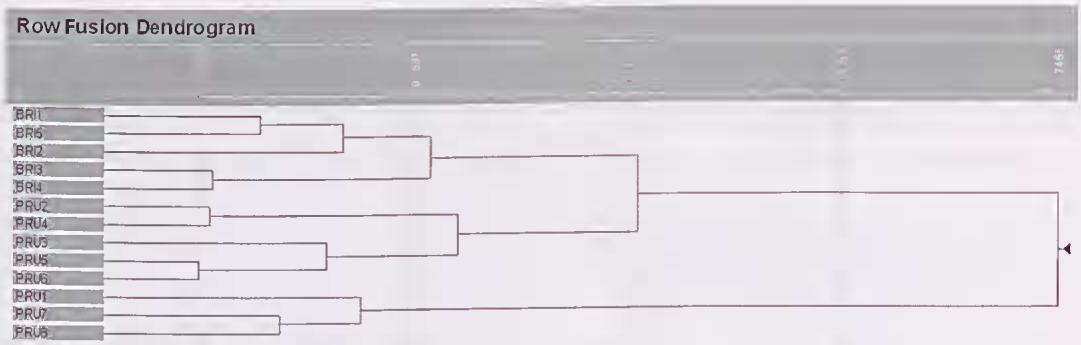

Figure 2: Cluster analysis (GM UPGMA) of morphological data using computer-based linear analysis. Names refer to populations (see appendix 1).

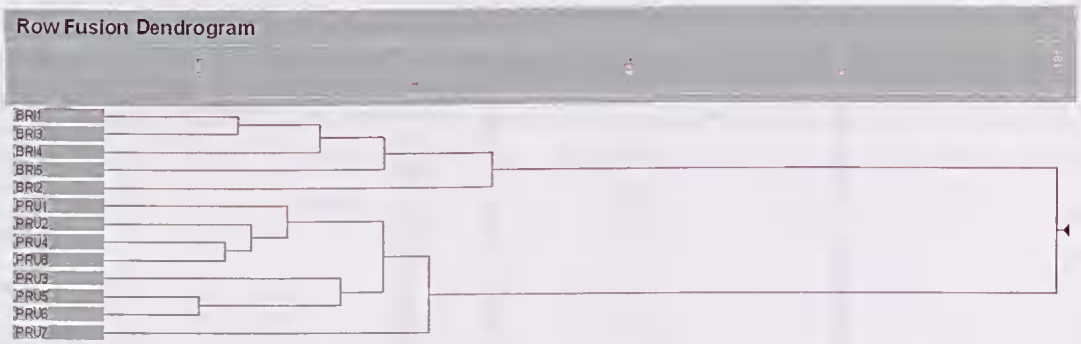

Figure 3: Cluster analysis (GM UPGMA) of TPS landmark analysis of leaf shape. Names refer to populations (see Appendix 1).

Use of the widest point on each side of the leaf blade as a landmark has been criticised by Jensen (1990) as not being based on genuine biologically homologous points, as these points may vary from leaf to leaf. However, they have been used as landmarks in this study as the leaves of the taxa investigated approximate to simple convex curves, making the widest point on each leaf unambiguous and clearly defined. This would be an inappropriate method if leaves were strongly lobed or toothed, with several possible locations on leaves being considered the widest point.

The landmark analysis was able to better discriminate between taxa than traditional length and width measures alone. It should be noted that tpsSuper is one of several methods available to analyse complex shapes. Other programs that use non-linear measures of shape were used during this study, but these produced results that were not readily reconciled with overall appearance of the specimens, or their provenance (data not shown). For further comments and comparisons of the various morphometric techniques see Premoli (1996), McLellan & Endler (1998) and Jensen *et al.* (2002).

Flavonoid analysis

Analysis of leaf phenolics shows differences in the presence of compounds in the two entities (Table 2). Major constituents have been shown to be consistently present in both entities, with variation shown in less common compounds: 'F' and 'N' being unique to the Briagolong variant and 'B', 'K', 'L' and 'M' being found only in typical *P. prunifolia*. Multivariate analysis (Fig. 4) of leaf flavonoid composition shows the Briagolong variant clearly separated from *P. prunifolia*, further supporting the separation of these two entities at specific rank. Flavonoid analysis may be a good method for validating the taxonomic status of other morphologically similar species of *Pomaderris*, but

Table 2. Presence/absence of flavonoid compounds in each population sample. Presence of compounds is denoted by X.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
bri1	X		X			X	X	X	X	X				X
bri2	X		X			X	X	X	X	X				X
bri3	X		X	X	X	X	X	X	X	X				X
bri4	X		X			X	X	X	X	X				
bri5	X		X	X	X	X	X	X	X	X				
pru1	X		X	X	X		X	X	X	X	X	X	X	
pru2	X		X	X	X		X	X	X	X	X	X		
pru3	X		X	X	X		X	X	X	X		X		
pru4	X	X	X	X	X		X	X	X	X	X			
pru5	X	X	X	X	X		X		X	X	X	X		
pru6	X	X	X	X	X		X		X	X	X	X	X	
pru7	X		X	X	X		X	X	X	X	X	X		
pru8	X		X	X	X		X	X	X			X		

clearly further work is required to test this across the genus. As far as we are aware it has not previously been employed in taxonomic studies of the genus.

Seedling trials

Although only a limited amount of seed was available for growth trials and results were not able to be statistically evaluated, seedlings of both typical *P. prunifolia* and the Briagolong variant, derived from several populations of each and grown under the same conditions, bred true to type and soon developed the characteristic shape and surface characteristics of the parent plants.

The strong separation shown in the above analyses, and the distinctness in the field when the two entities occur sympatrically and the uniform seedling characteristics suggest that the Briagolong variant is most appropriately recognised as a distinct species and is formally described below.

Taxonomy

Pomaderris briagolensis Messina *sp. nov.*

P. prunifolia *affinis sed foliis minoribus obovatis integris ad apicem truncatis vel emarginatis, pagina supra saepe glabra et nitida differt.*

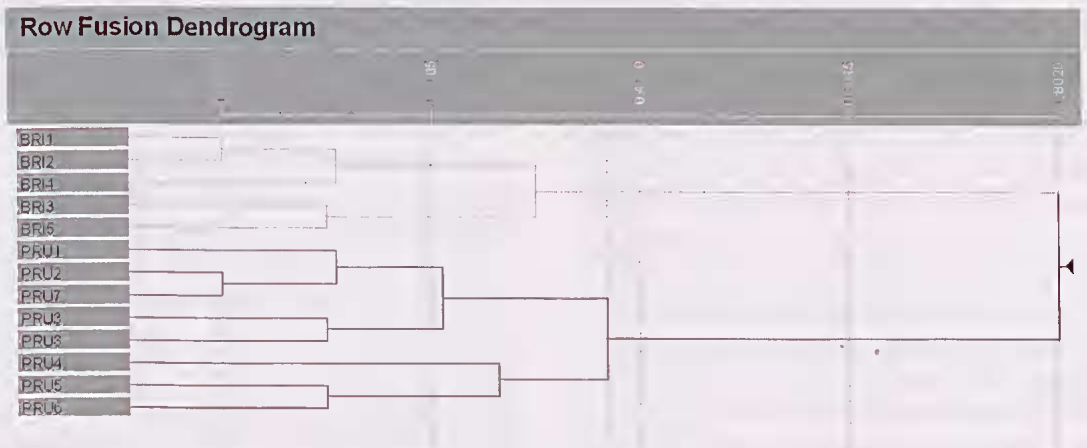

Figure 4: Cluster analysis (GM UPGMA) of leaf phenolic data. Names refer to populations (see Appendix 1).

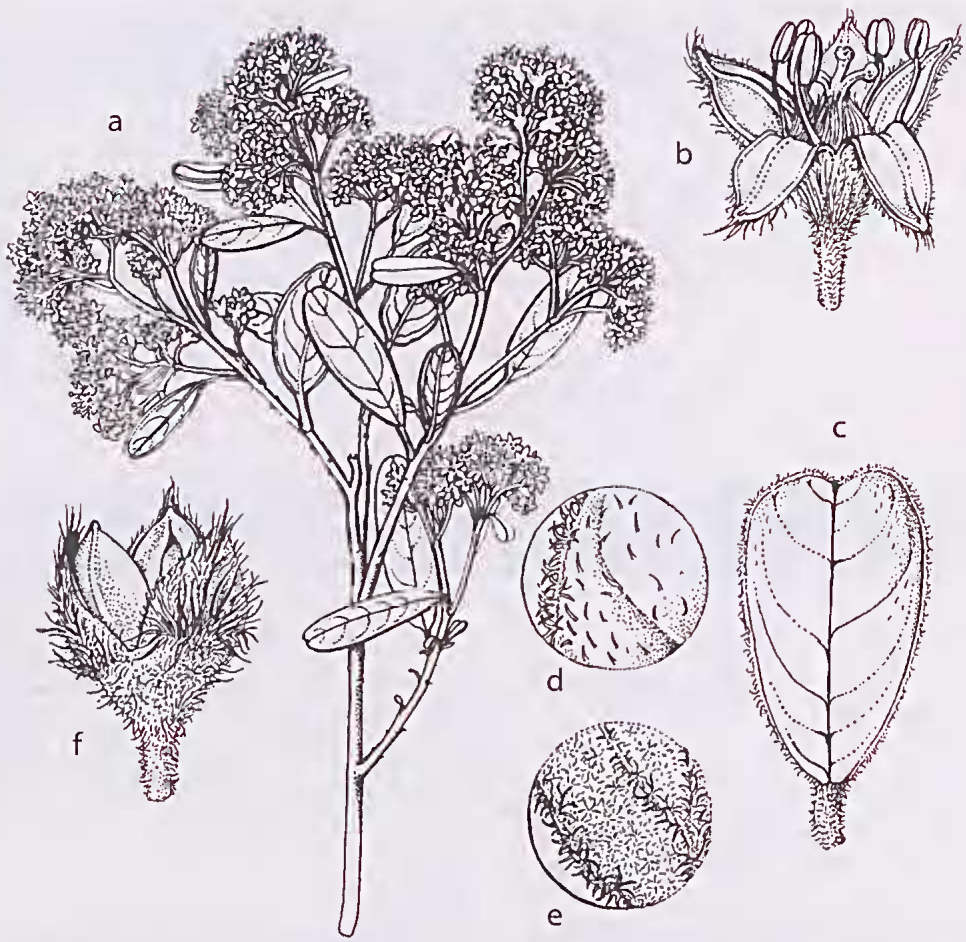

Figure 5: *Pomaderris briagolensis*. a flowering branch x 1; b flower x 8; c leaf, adaxial view, x 3; d leaf, adaxial indumentum, x 8; e leaf, abaxial indumentum, x 8; f fruit x 8 (a–e from *Kilgour 315*; f from *Walsh 6167* (MEL).

***Type*:** Victoria. Briagolong, Johnston's Flat campground, 8.x.2006, *A.Messina 42* (holo: MEL 2309916).

Shrub to 2 m high. *Branchlets* pubescent with dense rusty stellate hairs, indumentum persisting to older stems of current season's growth. *Stipules* linear-filiform to triangular 1–5 mm long, brown with whitish stellate hairs, commonly persisting after leaves have fallen. *Petiole* 4–9 mm long, pubescent with dense covering of rusty stellate hairs. *Lamina* obovate or c. oblong, 12–24 mm long, 6–10 mm wide; apex obtuse to truncate or slightly emarginate; base cuneate, margins entire; lamina penninerved with 5–10 pairs of lateral veins, slightly impressed on upper surface, raised on lower surface; upper surface glossy *in vivo*, glabrous to sparingly simple-hispid; lower surface of lamina densely covered with white stellate hairs, with larger rusty stellate hairs scattered across lamina and covering veins and margin. *Inflorescences* compact, paniculate, terminal or axillary, pyramidal, to 40 mm long and 30 mm diameter, with c. 20–60 flowers. *Pedicels* 1–2 mm long, pubescent with moderate to dense covering of whitish stellate hairs. *Bracts* 2–3 mm long, 0.75–1.8 mm wide, stellate-velutinous on outer surface, sparsely sericeous on inner surface, deciduous, falling before anthesis. *Hypanthium* cup-shaped, 1–1.5 mm long, densely covered with white stellate hairs and longer simple hairs. *Sepals* 1.5–2 mm long, 0.8–1 mm wide, acute at apex, outer surface covered by an indumentum of whitish stellate hairs and longer white and rusty simple hairs, inner surface glabrous and yellow with a raised midline.

Petals absent. *Disc* not apparent. *Stamens* alternate to sepals; filaments 1.5–2.2 mm long; anthers oblong, 0.5–1 mm long. *Ovary* inferior, 1–1.5 mm diam., summit pubescent. *Style* 0.7–1.7 mm long, 3-branched from near midway. *Schizocarps* ellipsoid, 2.5–3 mm long, 1.5–2 mm diameter, splitting into 3 nutlets at maturity; operculum on inner face of each nutlet membranous, c. half as long as nutlet, apex acute. *Seeds* flattened-ellipsoid, 1.5–2 mm long, shining brown with a small apical aril.

***Specimens examined*: VICTORIA**. Sportsman Ck Natural Feature Zone, 26.ix.19B4, *A.C.Beauglehole 77354* (CHR, HO, MEL); Freestone Ck Natural Feature Zone, 27.ix.1984, *A.C.Beauglehole 77394* (CANB, MEL); Banks of Freestone Ck, opposite Winkie Ck Tk, 14 km N of Briagolong, 24.x.19B2, *R.A.Kilgour 315* (MEL); Freestone Ck, approx. 2 km north of Briagolong, 24.iii.2005, *A.Messina 1* (LTB); Freestone Ck, approx. 20 km north of Briagolong, 24.iii.2005, *A.Messina 12* (LTB); Freestone Ck, approx. 21 km north of Briagolong, 24.iii.2005, *A.Messina 14* (LTB); Freestone Ck, approx. 22 km north of Briagolong, 24.iii.2005, *A.Messina 17* (LTB); Freestone Ck, approx. 20.5 km north of Briagolong, 24.iii.2005, *A.Messina 19* (LTB); Briagolong, Lees Tk, near Freestone Ck, 7.x.2006, *A.Messina 38* (MEL); Freestone Ck, 2 km N of Briagolong, 25.iv.19B9, *N.G.Walsh 2396* (CANB, MEL, NSW); Briagolong, beside Freestone Ck, 15.xii.2004, *N.G.Walsh 6167* (K, MEL).

***Distribution and conservation status*:** Known from populations along the Freestone Creek catchment near and upstream from Briagolong in eastern Victoria. It occurs in two Natural Feature Zones, with a known area of occupancy of approximately 15 km^2. Within this area it is locally abundant, with populations ranging from 2–300 individuals. It is recommended for classification as rare (2RC- V66, C67- Freestone Creek NFZ), applying the ROTAP criteria of Briggs and Leigh (1996). Applying the criteria of the IUCN Red List (IUCN 2001), *P. briagolensis* is recommended for classification as critically endangered (CR B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v)).

***Habitat*:** *Pomaderris briagolensis* occurs on steep slopes toward valley floors down to alluvial flats, on rocky soils consisting of gravel, sand, silt and clay (LCC 1982), in *Eucalyptus*-dominated woodlands and riparian shrublands. Associated species include *Eucalyptus bridgesiana* R.T.Baker, *E. macrorhyncha* F.Muell. ex Benth., *E. melliodora* A.Cunn. ex Schauer, *Acacia floribunda* (Vent.) Willd., *Cassinia longifolia* R.Br., *Kunzea ericoides* (A.Rich.) Joy Thomps. *sens. lat.* and *Prostanthera rotundifolia* R.Br. The altitude range is c. 70–200 m. At the site nearest the Briagolong township, *P. briagolensis* occurs with at least six other species of *Pomaderris* in a mixed shrubland. Communities in which multiple species of *Pomaderris* occur are known in several sites in south-eastern New South Wales and eastern Victoria. As at the Briagolong site, these sites share characteristics of relatively low altitude, rocky substrates and close proximity to watercourses within otherwise rather dry country.

***Notes*:** Originally included in *P. prunifolia* var. *prunifolia* due to its similar indumentum and distribution and general floral characters. However, it can be distinguished by its smaller, obovate, entire leaves which have obtuse to truncate or emarginate apices, and its generally glabrous and glossy upper leaf surface.

Of species that occur in eastern Victoria, the foliage of *P. briagolensis* is most similar in shape to that of *P. pauciflora* N.A.Wakef., but the leaves are larger, not distinctly recurved at the margins, lack simple hairs on lower leaf lamina, and the simple hairs on the upper lamina are absent or much sparser.

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Appendix 1. Collection sites of *P. prunifolio* and the Briagolong variant. All specimens held at LTB.

Species collected	Collector	Location	Lat/Long	Date
P. sp (bri1)	A. Messina 1 & N.G. Walsh	Briagolong – Freestone Creek	37°49'45" S 147°4'9" E	24/3/0S
P. sp (bri2)	A. Messina 12 & N.G. Walsh	Culloden – Freestone Creek	37°42'42" S 147°8'13" E	24/3/05
P. sp (bri3)	A. Messina 14 & N.G. Walsh	Culloden – Freestone Creek	37°42'26" S 147°8'30" E	24/3/05
P. sp (bri4)	A. Messina 17 & N.G. Walsh	Culloden – Freestone Creek	37°42'2" S 147°8'46" E	24/3/0S
P. sp (briS)	A. Messina 19 & N.G. Walsh	Culloden – Freestone Creek	37°42'43" S 147°8'27" E	24/3/05
P. prunifolio (pru1)	A. Messina S & N.G. Walsh	Briagolong – Freestone Creek	37°49'45" S 147°4'9" E	24/3/05
P. prunifolio (pru2)	A. Messina 20 & N.G. Walsh	Culloden – Freestone Creek	37°42'43" S 147°8'27" E	24/3/0S
P. prunifolio (pru3)	A. Messina 21	Warrandyte – Jumping Creek	37°44'20" S 145°14'39" E	7/4/05
P. prunifolia (pru4)	A. Messina 22	Warrandyte – Jumping Creek	37°43'56" S 14S°14'24" E	7/4/0S
P. prunifolio (pru5)	A. Messina 2S	Brisbane Ranges – Anakie Gorge	37°S1'42" S 144°16'14" E	12/S/0S
P. prunifolia (pru6)	A. Messina 26	Brisbane Ranges – Shaft Track	37°S0'S1" S 144°15'26" E	12/5/05
P. prunifolio (pru7)	A. Messina 27 & C. Nield	Mallacoota – Inlet National Park	37°30'36" S 149°41'35" E	3/6/05
P. prunifolio (pru8)	A. Messina 3S, P. Green & C. Nield	Lake Buffalo	36°42'44" S 146°40'10" E	1S/7/05